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Short Communication

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New Records of *Ixodes affinis* (Acari: Ixodidae) Parasitizing Avian Hosts in Southeastern Virginia

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Abstract

Ixodes affinis Neumann (Acari: Ixodidae) is a hard-bodied tick species distributed throughout much of the southeastern United States. Although *I. affinis* does not parasitize humans, it is a competent vector of *Borrelia burgdorferi* sensu stricto, the causative-agent of Lyme disease, and thus contributes to the enzootic maintenance of this pathogen. This study presents evidence of *I. affinis* parasitizing five new host passerine species. During 2012–2014, 1,888 birds were captured and examined for ticks, and 18 immature *I. affinis* were collected from 12 birds—six Carolina Wrens (*Thyrothorus ludovicianus*); two Brown Thrashers (*Toxostoma rufum)*; and one American Robin (*Turdus migratorius)*, Eastern Towhee (*Pipilo erythrophthalmus)*, Northern Cardinal (*Cardinalis cardinalis*), and White-throated Sparrow (*Zonotrichia albicollis).* Of 15 larvae and 3 nymphs collected, one nymph tested positive for *B. burgdorferi* DNA. *I. affinis* was found co-feeding on birds with immature *Amblyomma americanum* (L.)*, Ixodes brunneus* Koch*, Ixodes dentatus* Marx*, Ixodes scapularis* Say, and *Haemaphysalis leporispalustris* Packard. The results of this research provide a better understanding of *I. affinis* hosts and identify avian taxa that may play a role in the maintenance and dispersal of this tick species.

Key words: Avian tick, *Borrelia burgdorferi*, *Ixodes affinis*

As tick-borne disease incidence continues to rise in the United States, efforts to better understand the underlying ecological dynamics of tick-borne pathogens are increasingly urgent. Reported cases of Lyme disease are currently at an all-time high in the United States (CDC 2015); a more comprehensive understanding of the tick species and corresponding vertebrate hosts involved in the ecological cycle of *Borrelia burgdorferi* sensu stricto is crucial to long-term mitigation of Lyme disease.

Ixodes affinis Neumann (Acari: Ixodidae) is a hard-bodied tick species distributed throughout much of the southeastern United States (Oliver et al. 1987, Harrison et al. 2010). The primary vector of *B. burgdorferi* s.s. to humans is *Ixodes scapularis* Say in the eastern United States; however *I. affinis* is also a competent vector of this pathogen and is thought to play a role in the enzootic cycle of *B. burgdorferi* in the southeastern United States (Clark et al. 2001, 2002; Harrison et al. 2010; Maggi et al. 2010). *I. affinis* is undergoing a northward range expansion and has recently become established in the coastal plains of North Carolina (Harrison et al. 2010) and Virginia (Nadolny et al. 2011). *I. affinis* adults are known to feed on large mammals like white-tailed deer (*Odocoileus virginianus*), whereas immatures generally parasitize small mammal hosts, including mice, rats, and shrews (Oliver et al. 1987, Harrison et al. 2010). Little is known about the avian host preferences of this species, but. *I. affinis* has been reported to parasitize two avian species; one larva was found feeding on a Carolina Wren (*Thyrothorus ludovicianus*) (Oliver et al. 1987), and three larvae and one nymph were reported from a Swainson's Thrush (*Catharus ustulatus*) (Scott et al. 2012). The goal of this study was to determine additional passerine hosts of *I. affinis*, at the northern extent of its range in southeastern Virginia.

Materials and Methods

Tick Collection

Mist netting was conducted twice per month at each of five permanent sites and on an *ad hoc* basis ($N = 2$–14 total visits) at six additional sites from 2012 to 2014 in southeastern Virginia (Fig. 1). Up to ten 3 by 12-m mist nets were erected at dawn and checked every 30 min until bird activity quieted, usually at around 1100 hr. Birds captured were banded, morphometric data were taken, and individuals were examined for ticks around the head, in the ears, under the

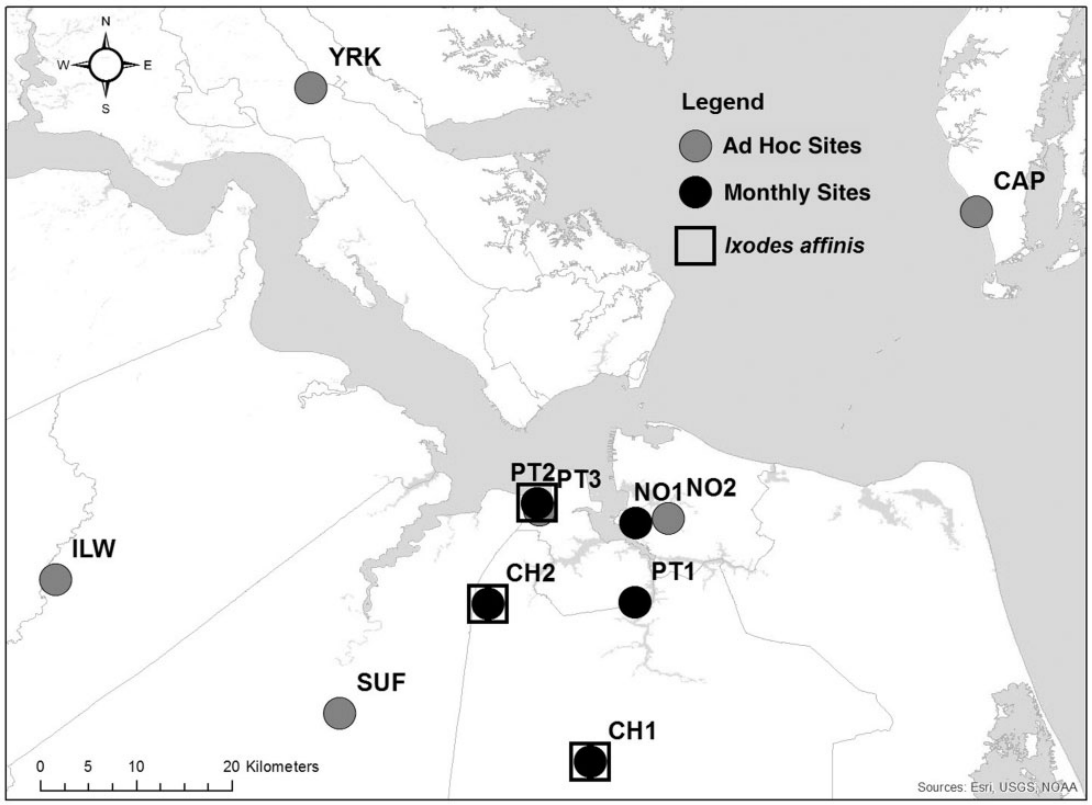

Fig. 1. Map of mist netting sites in southeastern Virginia. Sites marked with gray circles were sampled on an *ad hoc* basis ($N = 2–14$ visits over the course of the study), and sites marked with black circles were sampled approximately twice per month. Sites where *I. affinis* was collected from birds are marked with a square. The abbreviations at each site indicate the county or independent city in which the site was located: CAP = Cape Charles Co., CH = Chesapeake, ILW = Isle of Wight Co., NO = Norfolk, PT = Portsmouth, SUF = Suffolk, YRK = York Co. Counties or cities with more than one site are numbered.

wings, and around the cloaca. Ticks were removed with forceps, placed into vials, and stored in a −20°C freezer prior to morphological identification and processing. All birds captured were released immediately after banding and sample collection.

DNA Extraction

All ticks were identified initially to genus and life stage based on morphological features (Keirans and Clifford 1978). With the exception of rabbit ticks (*Haemaphysalis leporispalustris* Packard), which are easily identified to species morphologically (Clifford et al. 1961), all other ticks were subjected to DNA extraction to facilitate molecular testing. Prior to extraction, ticks were homogenized using equal volumes of 5- and 1-mm glass beads and subjected bead-beating on a Mini Beadbeater (BioSpec, Inc. Bartlesville, OK, USA). Extractions were carried out using the DNeasy Blood and Tissue Kit (Qiagen, Inc. Valencia, CA, USA) according to manufacturer's instructions. DNA was eluted in 50–100 µl of elution buffer.

PCR and Sequencing

To determine tick species, DNA extracts were subjected to a polymerase chain reaction (PCR) targeting a 454-bp fragment of the tick mitochondrial 16S rRNA gene. Primers used were 16S + 1 (5′-CTG-CTC-AAT-GAT-TTT-TTA-AAT-TGC-TGT-3′) and 16S-1 (5′-GTC-TGA-ACT-CAG-ATC-AAG-T-3′) (Nadolny et al. 2011). Reactions were carried out in 25 µl volumes using 2 × EconoTaq PLUS (Lucigen, Inc.), with 400 nM of each primer, 3 mM $MgCl_2$, and 5 µL DNA template. The thermocycling protocol consisted of 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 57°C for 45 s, and 72°C for 60 s, with a final extension at 72°C for 8 min. Products were visualized on a 1.5% agarose gel and purified using ExoSAP-IT (Affymetrix, Inc. Santa Clara, CA, USA). Sequencing reactions were performed using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequence similarities to tick 16S rRNA gene DNA sequences available in GenBank were identified by a BLAST search (http://blast.ncbi.nlm.nih.gov, last accessed 28 October 2015).

Pathogen Testing

To test for the presence of *Borrelia* spp. DNA in ticks, DNA extracts were subjected to a real-time PCR targeting the 23S rRNA gene of *Borrelia* spp. (Courtney et al. 2004). Although this assay will amplify *B. burgdorferi* DNA, it also amplifies other closely related *Borrelia* species, including *Borrelia bissettii*, *Borrelia andersonii*, and *Borrelia parkeri* (Courtney et al. 2004). Primers and probe used were Bb23Sf (5′-CGA-GTC-TTA-AAA-GGG-CGA-TTT-AGT-3′), Bb23Sr (5′-GCT-TCA-GCC-TGG-CCA-TAA-ATA-G-3′), and Bb23Sp-FAM (5′-FAM-AGA-TGT-GGT-AGA-CCC-GAA-GCC-GAG-TG-BHQ-1-3′). Reactions were carried out in 20 µl volumes using 2 × EconoTaq PLUS (Lucigen, Inc. Valencia, CA, USA), 700 nM each primer, 175 nM probe, 6 mM $MgCl_2$, and 2 µl template DNA. Thermocycler reaction conditions were 95°C for 5 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. *B. burgdorferi* B31 DNA was used as a positive control in all reactions. Sequencing to confirm *B. burgdorferi* in the *I. affinis* nymph was carried out on the *ospC* gene, as previously described (Bunikis et al. 2004).

Table 1. Avian hosts of immature *Ixodes affinis* (IA) in southeastern Virginia and observations of co-feeding with *Amblyomma americanum* (AA), *Ixodes brunneus* (IB), *Ixodes dentatus* (ID), *Ixodes scapularis* (IS), and *Haemaphysalis leporispalustris* (HL)

Host	Site	Month	IA		AA	IB	ID	IS		HL		Total
			Larvae	Nymphs	Nymphs	Larvae	Larvae	Larvae	Nymphs	Larvae	Nymphs	
American Robin[a]	PT2	July	1						1			2
Brown Thrasher[b]	CH2	June	3		2			1			1	7
Brown Thrasher[c]	PT2	Oct.	1							2		3
Carolina Wren	PT2	Aug.	1									1
Carolina Wren[a]	PT2	Aug.		1†						1		2
Carolina Wren[b]	PT2	Aug.		1					2, 1[c]	4	1	9
Carolina Wren[a]	CH2	Sept.	1							3	1	5
Carolina Wren[b]	CH1	Oct.	5				1	1		3	3	13
Carolina Wren[a]	CH2	Nov.	1							12		13
Eastern Towhee[a]	PT2	Oct.	1							14		15
Northern Cardinal	CH1	Sept.	1									1
White-throated Sparrow[a]	CH1	Dec.		1		1						2

[a]Birds parasitized by *I. affinis* and one other tick species; [b]birds parasitized by *I. affinis* and multiple other tick species; [c]one *I. affinis* nymph and one *I. scapularis* nymph positive for *Borrelia* species DNA.

Results

Immature *I. affinis* were collected from six species of passerine birds (Table 1) from three different sites (Fig. 1). Twelve of 1,888 birds sampled were parasitized by *I. affinis*. Avian hosts on which immature *I. affinis* were found included an American Robin (*Turdus migratorius*), two Brown Thrashers (*Toxostoma rufum*), six Carolina Wrens (*Thyrothorus ludovicianus*), an Eastern Towhee (*Pipilo erythrophthalmus*), a Northern Cardinal (*Cardinalis cardinalis*), and a White-throated Sparrow (*Zonotrichia albicollis*) (Table 1). Interestingly, *I. affinis* was frequently found co-feeding with other tick species, including immature *Amblyomma americanum, Ixodes brunneus, Ixodes dentatus, Ixodes scapularis, and H. leporispalustris*, with three instances of co-feeding with more than one other species (Table 1). *I. affinis* larvae were collected from birds from June through November, whereas *I. affinis* nymphs were collected from birds in August and December.

Of 18 *I. affinis* collected from avian hosts, a single nymph tested positive by real-time PCR for *B. burgdorferi*, based on sequencing using *ospC* gene primers. This nymph was found feeding on a Carolina Wren at a site in Portsmouth, Virginia. Of the five *I. scapularis* collected from birds, one nymph tested positive for *Borrelia* sp.; this *I. scapularis* nymph was found co-feeding with an *I. affinis* nymph on a Carolina Wren. The *I. affinis* nymph, however, was not infected with *Borrelia* sp.

Discussion

Ixodes affinis

We document *I. affinis* parasitizing five new avian host species and one avian host that had been reported previously (Table 2). Our study demonstrates that avian taxa may play a more important role as hosts of immature *I. affinis* than previously thought. Of the five additional avian host taxa identified in this study, one, the White-throated Sparrow, is not a year-round Virginia resident. This species winters in Virginia but travels north to breeding grounds in Canada in the spring (Falls and Kopachena 2010).

Migrating birds that move northwards through Virginia in the spring likely encounter *I. affinis* larvae and nymphs, life stages that are at peak abundance in the winter and spring (Oliver et al. 1987). Changes in *I. affinis* distribution have been linked to migrating birds (Harrison et al. 2010). The phenology of immature *I. affinis* in Georgia suggests that larvae are active throughout the fall and winter, and nymphs are active in winter through early summer (Oliver et al. 1987). Although the sample size of *I. affinis* is too low to establish phenology curves for Virginia, larvae were collected throughout the fall migration period, and nymphs were captured in summer and winter, suggesting a long activity period that may extend through fall or spring migrations. One study reported *I. affinis* larvae and nymphs parasitizing a Swainson's Thrush, a neotropical migrant, in Manitoba, illustrating the fact that birds are capable of transporting *I. affinis* thousands of kilometers during spring migration (Scott et al. 2012). Interestingly, the thrush was also infested with immature *I. scapularis* and *I. dentatus*, some of which tested positive for *B. burgdorferi*. While bird hosts may not be as important to the maintenance of *I. affinis* populations as mammal hosts, considering only 12 out of 1,888 (0.64%) birds served as *I. affinis* hosts, birds clearly have the potential to play a key role in *I. affinis* dispersal over large distances and may serve as hosts in areas where mammal abundance is low. Forests, especially pine plantations, are known to support lower abundance and diversity of small mammals in the coastal plain of Virginia than successional habitats (Bellows et al. 2001). These same forest fragments, however, support numerous bird species and are especially important stopover sites during migration (Kilgo et al. 1997).

Questing adult *I affinis* collected using flags or drags are easier to distinguish morphologically from other adult *Ixodes* spp. ticks, including *I. scapularis* (Nadolny et al. 2011). We encourage researchers, however, to remain vigilant for the presence of *I. affinis*

Table 2. Published avian host records of *I. affinis* nymphs (N), and larvae (L) in the United States and Canada

Common name	N	L	Location	Citation
Passeriformes				
Carolina Wren	X	X	Georgia	Oliver et al. 1987
Swainson's Thrush	X	X	Manitoba	Scott et al. 2012
American Robin		X	Virginia	This study
Brown Thrasher		X	Virginia	This study
Eastern Towhee		X	Virginia	This study
White-throated Sparrow	X		Virginia	This study
Northern Cardinal		X	Virginia	This study

No adults have been found parasitizing birds.

on avian hosts in the Mid-Atlantic, and use the best available molecular methods to identify engorged immature ticks removed from birds. Identification of engorged immature *Ixodes* spp. ticks using morphological characteristics is challenging at best, and misclassification is likely in studies relying solely on this method of identification.

Implications for *B. burgdorferi*

I. affinis is the primary maintenance vector of *B. burgdorferi* s.s. in the southeastern United States (Clark et al. 2001, 2002; Harrison et al. 2010; Maggi et al. 2010). As *I. affinis* arrives in northern states where *I. scapularis* and *B. burgdorferi* s.s. are already established, amplified transmission between shared reservoir hosts may increase the pathogen abundance in both tick species, increasing the risk of infected *I. scapularis* transmitting *B. burgdorferi* to humans (Nadolny et al. 2011). Many hosts of immature *I. affinis* are susceptible to infection by *B. burgdorferi*, including birds and small mammals that also host *I. scapularis* (Clark et al. 2002, Rudenko et al. 2013). We found *I. scapularis* co-feeding with *I. affinis* as larvae and nymphs on three avian species. We also documented *I. affinis* co-feeding with *I. dentatus* and I. *brunneus*, although it is unclear whether these two species are competent vectors of *B. burgdorferi* (Anderson et al. 1989).

Overlapping phenologies of nymphs and larvae of both *I. affinis* and *I. scapularis* in the spring and fall provide multiple opportunities for host sharing. *I. scapularis* immatures are known to quest throughout the summer months in the Mid-Atlantic (Hofmeister et al. 1999), and may parasitize birds during spring or fall migrations. The presence of co-feeding immature *I. affinis* and *I. scapularis* on possible avian reservoirs for *B. burgdorferi* strengthens the hypothesis that spillover from one tick species to another is likely in areas where tick species co-occur. Transmission via co-feeding is possible whenever infected nymphs are feeding alongside other naïve nymphs or larvae. Although both birds that were hosts to *Borrelia* sp.-infected nymphs in this study were Carolina Wrens, this does not indicate that wrens are necessarily competent reservoirs for *B. burgdorferi*. Additional study is warranted to determine which avian species are competent *Borrelia* sp. reservoirs, and may spread infection between *I. affinis* and *I. scapularis*.

Differences in the questing behavior of northern and southern *I. scapularis* ticks may have resulted in fewer human cases of Lyme disease in southern regions than in more northerly areas within the range of *I. scapularis* (Arsnoe et al. 2015). As the range of *I. affinis* moves northward, however, the addition of this second competent vector within areas occupied by northern *I. scapularis* may amplify the number of human cases. We have documented *I. affinis* parasitizing many more avian hosts than was previously thought. The ability to parasitize a range of avian hosts may have implications for the rate at which *I. affinis* moves northwards, and the types of habitat it can invade. After arrival and establishment, the effects on human risk from amplified levels of *B. burgdorferi* in the sylvatic cycle are unclear.

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